

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY  
DEPARTMENT OF PESTICIDE REGULATION  
MEDICAL TOXICOLOGY BRANCH

LINDANE  
(Gamma Hexachlorocyclohexane)

SB 950-073, Tolerance # 133  
Chemical Code 359

December 11, 1986  
Revised 10/24/88, 1/17/90, 1/04/91, 2/18/93, and 11/30/98

I. DATA GAP STATUS

|                         |   |
|-------------------------|---|
| Chronic toxicity, rat:  | No data gap, no adverse effect  |
| Chronic toxicity, dog:  | No data gap, no adverse effect  |
| Oncogenicity, rat:      | No data gap, possible adverse effect†.                                  |
| Oncogenicity, mouse:    | No data gap, possible adverse effect                                    |
| Reproduction, rat:      | No data gap, possible adverse effect                                    |
| Teratology, rat:        | No data gap, no adverse effect  |
| Teratology, rabbit:     | No data gap, no adverse effect  |
| Gene mutation:          | No data gap, no adverse effect  |
| Chromosome aberrations: | No data gap, possible adverse effect indicated (in an inadequate study) |
| DNA damage:             | No data gap, no adverse effect  |
| Neurotoxicity:          | Not required at this time   |

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† An acceptable rat combined study indicates no adverse effects, however a 1977 NCI study was judged by DPR to indicate oncogenicity in liver, pituitary, and thyroids in another rat strain.

All records for the above study types through Record No. 124423 (Document No. 133-066) were examined. This includes all relevant studies indexed by DPR as of 11/30/98.

Revised by Shimer & Davis, 10/24/88; M. Silva, 1/17/90, C. Aldous and J. Gee, 1/4/91, H. Green and Aldous, 2/18/93, and Aldous, 11/30/98. File name: T981130.wpd

In the 1-liners which follow:

\*\* indicates an acceptable study.

**Bold face** indicates a possible adverse effect.

These pages contain summaries only. Individual worksheets may identify additional effects.

## II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

## COMBINED, RAT

**\*\*133-060 091997** "Lindane: Combined Oncogenicity and Toxicity Study by Dietary Administration to Wistar Rats for 104 Weeks", (S.J. Amyes, Life Science Research Limited, Eye, Suffolk, England, Report # 90/0839, 11/26/90). Lindane technical, 99.5%-99.7% purity, was fed in the diet at 0, 1, 10, 100, and 400 ppm. Estimated achieved dosages were 0.05, 0.45, 4.5, and 18.7 mg/kg/day for males, and 0.06, 0.57, 5.6, and 23.1 mg/kg/day for females. In the toxicity phase, 15 Wistar rats/sex/group received treated diet for 30 days, 26 weeks, or 52 weeks. A fourth series (same group sizes) was treated for 52 weeks and then maintained on control diet for 26 weeks prior to sacrifice. The oncogenicity study used 55 animals/sex/group treated for 104 weeks. In all toxicity and oncogenicity phase groups, 5 rats/sex/group were allocated for lindane tissue level assays. NOEL = 1 ppm in males (hyaline droplets in proximal tubules, and periadrenal hepatocytic hypertrophy at 10 ppm upwards). For reasons noted in the discussion section VI. B., kidney findings due to lindane have been shown to be associated with an  $\alpha_2$ -globulin mechanism, not considered relevant to humans. Common kidney findings in males at 100 and 400 ppm also included cortical tubule necrosis and regeneration, interstitial chronic nephritis, and papillary mineralization. Kidneys were not a target tissue in females. Periadrenal hepatocytic hypertrophy incidence was markedly increased at 100 and 400 ppm in both sexes. Liver changes were largely reversible, particularly in males. Thus the NOEL of 10 ppm in females is an appropriate "working NOEL" for relevant chronic effects in males also. Pheochromocytomas were marginally elevated in 400 ppm males. This was considered a possible adverse effect in the initial DPR review, hence DPR requested historical control data for adrenals in males of this strain, plus histopathology on all rats in intermediate groups. Requested data were provided in Document No. 133-066, Record Nos. 124419 to 124423. The lack of any compelling pattern of tumor incidence in the primary report as amended, coupled with the body of historical data showing this to be a common tumor type, indicate that the small numerical increase in pheochromocytoma incidence among 400 ppm males was incidental. Other findings included increased mortality in 400 ppm females and possibly also in 100 and 400 ppm males; body weight decrements after about 1 yr in both sexes at 400 ppm; increased incidence of convulsions in 400 ppm females; small reductions in RBC parameters (Hb, HCT, and RBC count) in 400 ppm males and females (with indications of a reciprocal increase in platelet counts in 100 and 400 ppm males); blood chemistry changes, generally limited to 400 ppm groups, which appeared to be treatment-related in males [increased calcium (minimum extent), and increased total protein], females (increased cholesterol and increased urea), or both (increased inorganic phosphorus and decreased albumin/globulin ratio); elevated organ weights (kidneys in 100 to 400 ppm males, and livers in 400 ppm males and females). Study is **acceptable with no adverse effects**. (H. Green and C. Aldous, 2/18/93; Aldous, 11/30/98).

133-054 074578 Interim report for 060:091977, above.

133-066 124423 Amyes, S. J., [Addendum to Document #133-060, Record #091997], original study title: "Lindane: Combined Oncogenicity and Toxicity Study by Dietary Administration to Wistar Rats for 104 Weeks", Pharmaco-LSR Ltd.; current submission dated 6/2/93. Laboratory Study #: LSR 93/0353 [an addendum to LSR Report # 90/0839]. The original report data suggested a possible increase in pheochromocytomas among high dose males. Adrenals of term survivors of intermediate dose groups were not routinely examined in the original analysis. This supplement provides adrenal histopathology of these rats, and an analysis of the total data for male rat pheochromocytomas. Incidence of pheochromocytoma in high dose males is not

sufficiently different from controls and other dose groups to attribute to treatment. Aldous, 11/30/98.

133-066 124422 (Various authors) Title of compilation of reprints was "Historical control data to support the study: "Lindane: Combined Oncogenicity and Toxicity Study by Dietary Administration to Wistar Rats for 104 Weeks", compilation prepared June, 1993. Ranges of adrenal pheochromocytoma incidences by study were as follows:

Bomhard, E., E. Karbe, and E. Loeser, "Spontaneous tumors of 2000 Wistar TNO/W.70 rats in two-year carcinogenicity studies", *JEPTO Z*:35-52 (1986). Incidences were highly variable: range in males was 0% to 38.4%, with 62 out of 962 males affected overall (6.4%).

Roe, F. J. C. and A. Bär, "Enzootic and epizootic adrenal medullary proliferative disease of rats: Influence of dietary factors which affect calcium absorption", *Human Toxicol.* **4**: 27-52 (1985). Investigators found pheochromocytoma incidence to be variable, and highly influenced by diet. Some earlier studies were cited in which the majority of Wistar-derived males were affected.

Wester, P. W., I. I. Krajnc, F. X. R. van Leeuwen, J. G. Loeber, C. A. van der Heijden, H. A. M. G. Vaessen, and P. W. Helleman, "Chronic toxicity and carcinogenicity of bis(tri-n-butyltin)oxide (TBTO) in the rat", *Fd. Chem. Toxicol.* **28**:179-196 (1990). Unique contribution of this report is a positive pheochromocytoma response in both sexes, in conjunction with increased pituitary tumors in both sexes and increased parathyroid tumors in treated males. Concurrent control incidence of pheochromocytoma in males was 16/50.

Vandenberghe, J. "Life-span data and historical data in carcinogenicity testing in Wistar rats Crl:(WI)BR" (Charles River Deutschland, 1990). This was the most detailed of the enclosed historical control submissions. Incidences among 4 groups of 50 males housed up to 108 weeks (groups a and b) or up to 136 weeks (groups c and d) ranged from 8% to 22% (p. 31).

Collectively, these studies show pheochromocytoma incidence to be a comparatively common and variable event in male Wistar rats. No worksheet. Aldous, 11/24/98.

#### CHRONIC TOXICITY, RAT

133-017 034163-034165, 910376 Limited individual histopathology data from a 1-year rat inhalation study completed prior to Aug. 1951. No adverse effects indicated. No CDFA review. Aldous, 1/3/91.

133-017 034162 Three-page report of a chronic dietary study in rats. Study was completed in 1950, and employed only 10/sex/group. No useful data: no CDFA review. Aldous, 1/3/91.

#### CHRONIC TOXICITY, DOG

**\*\*039 059698 "Lindane Toxicity Studies in Beagle Dogs (Initial Studies and Dietary Intake for 104 Weeks)" (Huntingdon Research Centre, 9/2/71) Lindane (purity not stated) was fed for 104 weeks to 4 beagle dogs/sex/dose level at 0, 25, 50 and 100 ppm. NOEL = 50 ppm (elevated serum alkaline phosphatase levels and macroscopic liver anomalies). [Initial report (014 910293) was evaluated as "insufficient information for assessment" since alternate pages were missing: this was corrected in the present submission (Schreider 4/24/85)]. **No adverse effects; Acceptable.** Shimer and Davis 9/9/88.**

039 059697 "Lindane Toxicity Studies in Beagle Dogs (Initial Studies and Dietary Intake for 50 Weeks)". Interim report for 059698. Davis 9/9/88.

014 910293 Initial report submitted to CDFA, evaluated as "insufficient information for assessment" since alternate pages were missing. Schreider 4/24/85.

013 910274 "Lindane Toxicity Studies in Beagle Dogs. Dietary Intake (200 ppm) for 32 Weeks" (Huntingdon Research Center, 1/22/71). Lindane was tested at 200 ppm in the diet for 18 months in beagle-dogs; 4 dogs/sex/dose group, in a pilot study to determine if high dose (200 ppm) would result in signs of toxicity. **No adverse effects; Supplemental.** Schreider 4/17/85.

#### ONCOGENICITY, RAT

**040 059702** "Bioassay of Lindane for Possible Carcinogenicity." (Gulf South Research Institute, NCI, DHEW Publication No. 77-814, 1977) Lindane (100%) from 2 suppliers was fed to 50 Osborne-Mendel rats/sex/group in the diet for 80 weeks, followed by untreated diet for 30 weeks. Ten/sex were concurrent controls. Dose levels were reduced once on study for males and twice for females to yield time-weighted averages of 236 and 472 ppm for males and of 135 and 270 ppm for females. **Possible adverse effect:** neoplastic nodules in livers of both treated groups of both sexes, pituitary adenomas and carcinomas in both treated male groups and pituitary adenomas in both treated female groups, thyroid adenomas and carcinomas in both treated male groups and thyroid adenomas in both treated female groups. NOEL not established. **Unacceptable:** few control rats, only two dose levels, dose levels changed on study, MTD not reached, dosing period too short. Shimer and Davis, 7/26/88.

EPA one-liner: Oncogenic NOEL > 640 ppm (MDT) (sic). (Dosage levels = 80, 160, 320, 640 ppm) (sic)

038 059695 "Lindane Bioassay Studies and Human Cancer Risk Assessment" by Vesselinovitch and Carlborg (Toxicologic Pathology 11:12-22, 1983; University of Chicago). This review article of both mouse and rat oncogenicity studies includes re-evaluations of histology slides from four studies, a review of six other publications, and a human cancer risk assessment. An expanded version forms the Response of the Centre International d' Etudes du Lindane to EPA's preliminary Notice of Determination and Position Document 2/3 on Lindane (CDFA Volume 009). **No adverse effects** - Authors assert that 9 of the 10 studies were negative; benign liver tumors were found in males in the 10th study; risk to humans is considered insignificant. The Medical Toxicology reviews do not agree with these conclusions. **SUPPLEMENTAL STUDY.** Shimer and Davis 7/29/88.

#### ONCOGENICITY, MOUSE

SUMMARY: Considering all of the oncogenicity studies which Medical Toxicology has reviewed as well as the Vesselinovitch documents and the EPA documents, there appears to be a pattern of liver toxicity including hepatocarcinogenicity. Although none of these studies is acceptable and a NOEL has not been established, there is no obvious benefit to conducting another study. We consider the data requirement to be filled and a possible adverse effect to be identified. (Davis, 10/24/88).

**040 059703** "Bioassay of Lindane for Possible Carcinogenicity." (Gulf South Research Institute, NCI, OHEW Publication No. 77-814, 1977) Lindane (100%) from 2 suppliers was fed to 50 B6C3F1 mice/sex/group in the diet at 80 or 160 ppm for 80 weeks, followed by untreated diet for 10-11 weeks. Ten/sex were concurrent controls. **Possible adverse effect:** liver neoplasms in both sexes. NOEL not established. **Unacceptable, not upgradeable:** too few control mice, only two dose levels, MTD not clearly established. Shimer and Davis, 7/27/88.

EPA one-liner: Oncogenic NOEL > 160 ppm (MDT) (Dosage levels = 80, 160 ppm)

014 910386 "Carcinogenicity Study of Lindane in the Mouse" by Weisse and Herbst, Dept. Experimental Pathology and Toxicology, C. H. Boehringer Sohn, Germany; Publication (Toxicology 7:233-238, 1977) Lindane (99.5%) tested at 0, 12.5, 25, and 50 ppm (corresponding to 0, 2.1, 4.1 and 8.2 mg/kg for males and 0, 2.0, 3.9, and 7.8 mg/kg for females) in the diet for 80 weeks in NMRI (SPF) mice; 50 mice/sex/treated group and 100 mice/sex for controls; No adverse effects: no chronic toxicity or oncogenicity; UNACCEPTABLE-dose levels far below MTD, no analysis of diet, incomplete study parameters. Schreider 4/25/85, Davis 8/12/88.

038 59696 "Testing of the Substance Lindane for Cancerogenic Effects in Mice Using Oral Administration-Duration 80 Weeks" (C.H. Boehringer Sohn Ingelheim am Rhein, 4-75). Supplemental to 910386-protocol information, investigators' signature page, test material characterization, mean body weight and food consumption data, dates of deaths and autopsy findings, individual autopsy and microscopic findings at termination, summary of tumor findings. Shimer and Davis 8/12/88.

**040 059701** "The Toxicology of Dieldrin (HEOD). II. Comparative Long-term Oral Toxicity Studies in Mice with Dieldrin, DDT, Phenobarbitone, beta-BHC and gamma-BHC" by Thorpe and Walker. (Fd. Cosmet. Toxicol. 11:433-442, 1973). A comparative study of the response of CF1 mice to various chemicals capable of inducing microsomal enzymes in mammalian liver cells. Based on the results of range-finding tests, groups of 30 of each sex were fed diets with 10 ppm dieldrin (> 99%), 100 ppm DDT (> 99.5%), 500 ppm sodium phenobarbitone (> 98%), 200 ppm beta-BHC (> 99X), or 400 ppm gamma-BHC (> 99.5%) for 110 weeks. Negative controls were 45 mice/sex. **POSSIBLE ADVERSE EFFECT:** elevated frequencies of hyperplastic foci and tumors in the livers of both sexes for all treated groups. For lindane (gamma-BHC) the elevations were significant at the  $p < 0.01$  level. The incidences of tumors in other tissues were lower than control incidences for most treated groups including the lindane (gamma-BHC) group. **SUPPLEMENTAL STUDY.** Shimer and Davis 7/25/88.

**040 059655** "Induction of Hepatoma in Mice by Benzene Hexachloride" by Hanada et al. (Gann 64:511-513, October, 1973, Dept. of Pathology, Osaka University School of Medicine). Ten or 11 dd mice/sex/group were fed a control diet or one of four test materials (crude BHC, alpha BHC, beta BHC, and gamma BHC = lindane) at 100, 300, and 600 ppm for 32 weeks and then returned to the basal diet for 5 or 6 weeks, at which time they were sacrificed. **POSSIBLE ADVERSE EFFECT FOR THE CRUDE EXTRACT, ALPHA ISOMER, AND GAMMA ISOMER (LINDANE):** liver tumors, with higher frequencies in males. NOEL = 100 ppm. **SUPPLEMENTAL STUDY.** Shimer and Davis 7/22/88.

040 059699 "Histologic and Ultrastructural Studies on the Hepatocarcinogenicity of Benzene Hexachloride in Mice" by Ito, N., et al., (J Natl Cancer Inst 51:817-826, 1973) Groups of 20 or 40 male strain dd mice were fed alpha, beta, gamma, or delta BHC for 24 weeks at 100, 250, and 500 ppm singly and in various combinations to test for interactions. **NO ADVERSE EFFECTS**

FOR LINDANE (GAMMA ISOMER) - No compelling evidence for oncogenicity from lindane alone or in combination with other isomers (NOEL ~ 500 ppm). Hepatocarcinogenicity from the alpha isomer (NOEL = 250 ppm). **Supplemental study.** Shimer and Davis 7/22/88.

**040 059700** "Carcinogenicity of Benzene Hexachloride (BHC)" by Nagasaki, H., et al. of Nara Medical University, Japan (Pages 343-353 of "Topics in Chemical Carcinogenesis", Ed. Nakahara *et al.*, Univ. Tokyo Press, Tokyo, 1972). Twenty male dd mice per group were fed a diet with BHC (mixture of isomers) at 6.6, 66.0, and 660.0 ppm for 24 weeks. Fourteen male dd mice were fed a basal diet alone, as negative controls. **Possible adverse effect:** BHC elicited liver tumors in all 20 high dose mice but not in other mice. Alpha and beta isomers accumulated in the livers of all treated groups. Other organs were unaffected. **Supplemental study.** Shimer and Davis 7/25/88.

012 910381 "Carcinogenicity Study with Lindane in Mice. Electron Microscopical Investigation of Livers", (C. H. Boehringer Sohn, 8/5/76). Liver samples from four NMRI mice/sex/group, fed 0, 12.5, 25, or 50 ppm for 80 weeks in an oncogenicity study, were examined by electron microscopy. No adverse effects: results were completely negative. Supplemental study. Davis 9/22/88.

133-012 910402 Evidently part of 910381, above. No separate review. Aldous, 1/3/91.

See also 038 059695 under ONCOGENICITY, RAT.

133-027 019912 "Points concerning Lindane Position Document 2/3 submitted to the Scientific Advisory Panel by Paper Products, Inc." This is mainly a rebuttal to risk assessment evaluations by U.S. EPA, with some references to mouse studies completed prior to this record (Aug. 1980). Primary references are to Dr. Vesselinovitch: presumed to be the same statements noted by Dr. Davis in the 10/24/88 "Summary" statement on mouse oncogenicity. No further review of this record is necessary. Aldous, 2/8/93.

## REPRODUCTION, RAT

013 910398 "Effect of Lindane on Reproductive Function of Multiple Generations in the Rat." (Huntingdon Research Centre, Report # 4289 71/445, 2/16/72) Lindane (Batch No. 6801/403, \$ 99.0%) tested at 0, 25, 50 and 100 ppm in the diet in a 3-generation study in Charles River CD rats; 10 male and 20 female rats/dose group for all generations; increase in liver weight in F3 pups at 100 ppm; NOEL = 50 ppm; **NO ADVERSE EFFECT; INCOMPLETE; UNACCEPTABLE** (dose levels not high enough, insufficient histopathology and necropsy, unknown clinical observation intervals, and too few males). Schreider 4/22/85, Davis 9/21/88.

EPA One-Liner: Core Minimum, 1/23/89.

012 910275 "Effect of Lindane on Reproductive Function of Multiple Generations in the Rat. (1) The Determination of Dietary Concentration of Lindane. (2) Residues of Lindane in Rat Tissues" (Huntingdon Research Centre, 1/27/72). Describes the methods (gas liquid chromatography) and results for lindane analyses in the rat diet and selected tissues of weanlings. Davis 9/21/88.

Rebuttal in 038 provides information on test material and other questions from the original CDFA review.

133-027 910401 Published report, briefly summarizing study 013:910398, above.

**133-013 034177** Naishtein, S. Y., and Leibovich, D.L., "Effect of small doses of DDT' (-hexachlorocyclohexane and their mixtures on the sexual function and embryogenesis of rats", Gig. Sanit. 36:19-22 (1971). Abstract in English of Soviet report. Rats were said to have had altered estrus cycles, decreased numbers of fetuses, increased numbers of dead fetuses, and lower body weight gains of fetuses than controls. No data are available for evaluation. No CDFA review is necessary, since more documentable studies are available. Aldous, 1/3/91.

**\*\*133-062 112033**, "Lindane: Reproductive performance study in rats treated continuously through two successive generations", V.C. King (Study Director), Life Science Research Limited, Eye, Suffolk, England, Report # 91/CIL004/0948, 9/12/91). Lindane technical, Batch No. DA433, purity 99.5 to 99.7%. Two generation study, 1 litter/generation, with test article administered in the diet at 0, 1, 20, and 150 ppm to 30 CD® rats (Charles River, Margate, England) per sex per group. Treatment began 10 weeks prior to mating. Estimated dose levels for low, medium, and high doses, respectively during week 1 of the F0 pre-mating period were 0.14, 2.77, and 19.7 mg/kg/day for males and 0.13, 2.75, and 19.5 mg/kg/day for females. Corresponding values at week 10 of premating were 0.05, 0.99, and 7.42 mg/kg/day for males and 0.06, 1.20, and 8.74 mg/kg/day for females. F1 pre-mating doses were comparable. Parental NOEL = 1 ppm [increased incidence of periacinar hepatocytic hypertrophy in males (slight degree); typical "2u-globulin-related kidney effects in males, such as hyaline droplets in proximal tubules, tubular necrosis, regeneration, tubular casts, and chronic interstitial nephritis]. Neither of the above findings appears important to human safety, since the liver effect was shown to be reversible after much higher exposures in the combined study (DPR Record No. 091997), and the kidney effects are species- and sex-specific. Parental NOAEL = 20 ppm (slight body weight decrements in females during gestation, and in F1 males during the growth period; periacinar hepatocytic hypertrophy in both sexes; apparent hydronephrosis effect in males). Reproductive NOEL = 20 ppm [reduced neonatal pup survival (largely due to total litter losses); slightly reduced pup growth rate; slightly slower pup development (delays in hair growth and tooth eruption)]. **Acceptable, with a "possible adverse effect"** (decreased pup survival). H. Green and C. Aldous, 2/5/93.

113-063 113460, is an adverse effects disclosure (reduced pup survival at 20 and 150 ppm), dated 27 February 1992, for Record # 112033.

## TERATOLOGY STUDIES

**COMMENTS ON THE TERATOLOGY CATEGORIES:** While none of the following six studies is individually acceptable, this reviewer feels that collectively they are sufficient to fill the data gaps for both teratology categories. Each of the studies was criticized for having too few pregnant dams, but taken together they are more than adequate. Dose level selection was also questioned in five of the six studies. The study in which rabbits were dosed by gavage did not use high enough dose levels, but the injection study in rabbits did produce maternal toxicity. Similarly, the injection study in mice was criticized for inadequate dose levels but the gavage study in mice produced maternal toxicity. Both studies in rats produced maternal toxicity. The studies, done in three different laboratories, with two different routes of exposure, and three different species, were consistently negative. Further studies are unlikely to provide new information. Davis 9/22/88.

## TERATOLOGY, RAT

014 910388 "Teratology Study in Rats-Lindane (Gamma Benzene Hexachloride, USP)." (Hazleton Laboratories America Inc., 7/13/76). Lindane (purity unspecified) tested at 0, 5, 15 and 30 mg/kg in corn oil by subcutaneous injection on Days 6-15 of gestation in Charles River Sprague Dawley rats; 15-18 pregnant rats/dose group; toxicity observed at 30 mg/kg; decrease in food consumption (Days 6-11), body weight gains and increase in mortality (2/20) and clinical signs (tremors, excitability); extra ribs only present in the presence of maternal toxicity; **No adverse effects, incomplete, unacceptable:** (no purity assay of test compound, no analyses of dose solutions, not enough pregnant animals, no justification of route of administration, insufficient visceral data, no quality assurance statement). Schreider 4/24/85.

013 910394 "Effect of Lindane on Pregnancy of the Rat." (Huntingdon Research Center, 12/3/71) Lindane (> 99.0%) tested at 0, 5, 10, 20 mg/kg in 0.5% aqueous carboxymethylcellulose mucilage by oral gavage on Days 6-15 of gestation in CFY rats. Decrease in body weight gain and food consumption at 10 and 20 mg/kg; Maternal NOEL = 5 mg/kg; dose-related increase of 14th rib at all doses; significant at 20 mg/kg (but within historical control range); Developmental NOEL = 10 mg/kg; **No adverse effects, incomplete, unacceptable:** (not enough pregnant animals, no justification of dose levels, no dosing solution analysis, no individual fetal data, insufficient protocol information). Schreider 4/18/85, Parker 12/10/86, Davis 9/21/88.

Rebuttal in 038 provides information on test material and other questions from the original CDFA review.

#### TERATOLOGY, MOUSE

012 910390 "Lindane Testing for Teratogenic Effects in Mice Following Subcutaneous Injection." (E. Merck-Darmstadt, 1/28/72) Lindane (purity unspecified) tested at 0 and 6 mg/kg in 0.5% CMC-mucilage by subcutaneous injection (10 ml/kg) in two groups of NMR-EMD-SPF mice; group I injected daily on Days 6-15 and group II on Days 11-13 of gestation; 25 females/dose group; increased number of runts in group II at 6 mg/kg; NO ADVERSE EFFECT; INCOMPLETE; UNACCEPTABLE (purity of test compound is not specified, not enough pregnant animals in group II, no justification of route of exposure, only one dose level tested, no justification of dose level, no clinical observations, incomplete necropsy/histopathology study, no soft tissue data on fetuses). Schreider 4/25/85, Davis 9/21/88.

012 910392 "Lindane Testing for Teratogenic Effects in Mice Following Oral Administration." (E. Merck-Darmstadt, 5/4/72) Lindane (> 99.0%) tested at 0, 12, 30, 60, mg/kg in 0.5% aqueous carboxy methylcellulose mucilage by oral gavage (10 ml/kg) in two groups of NMRI-EMD-SPF mice; group I treated on Days 6-15 and group II on Days 11-13 of gestation; 25 females/dose group; toxicity observed at 60 mg/kg; decreases in activity, body weights and live fetuses; increase in mortality and abortions; NO ADVERSE EFFECT; no fetotoxic or teratogenic effects were observed until at high doses that caused extreme maternal toxicity; INCOMPLETE; UNACCEPTABLE (no dosing solution analysis, too few pregnant dams, high dose level too toxic). Schreider 4/25/85, Davis 9/21/88. Rebuttal in 038 provides information on test material and other questions from the original CDFA review.

#### TERATOLOGY, RABBIT

014 910389 "Teratology Study in Rabbits." (Hazleton Laboratories, 8/6/76). Lindane (purity unspecified) tested at 0, 5, 15 and 45/30 mg/kg (animals received 45 mg/kg on Days 6-9 and 30



mg/kg on Days 10-18 of gestation) in corn oil by subcutaneous injection from Days 6-18 of gestation in New Zealand white rabbits; 11-15 pregnant rabbits/dose group; high mortality at 45 mg/kg (12/13); dose related loss of body weight, decrease in food consumption and clinical signs at 15 and 45 mg/kg; embryo lethality at 45 mg/kg in presence of maternal toxicity; **NO ADVERSE EFFECT; INCOMPLETE; UNACCEPTABLE** (purity of compound, analysis of dosing solution, not enough animals, justification of dose levels, incomplete examination on fetuses for visceral and skeletal effects, no visceral effects were presented; no sex ratio was presented, excessive deaths at high dose, no justification of route of administration, no quality assurance statement). Schreider 4/24/85.

013 910396 "Effect of Lindane on Pregnancy of the New Zealand Rabbit." (Huntingdon Research Center, 12/1/71) Lindane (> 99.0%) tested at 5, 10 and 20 mg/kg in 0.5% aqueous carboxymethylcellulose mucilage by oral gavage on Days 6-18 of gestation in New Zealand rabbits; 13 animals/dose group. Report states increase respiration and drowsiness in all doses; however, no data presented. Preimplantation loss at 20 mg/kg; however, implantation occurs prior to initiation of dosing. Maternal NOEL <sup>™</sup> 20 mg/kg (Differences in food consumption and weight gain minimal); fetal loss increased with dose but was within historical range; abortions (one per dose group) at 10 and 20 mg/kg; increase in 13th ribs in fetuses and decreased ossification of sternebrae at 10 and 20 mg/kg but these are variable and minor changes which may indicate borderline fetotoxicity; Developmental NOEL <sup>™</sup> 20 mg/kg; **NO ADVERSE EFFECT; INCOMPLETE; UNACCEPTABLE** (too few pregnant dams, dose levels not high enough, no diet analysis). Schreider 4/19/85, Parker 12/10/86, Davis 9/21/88. Rebuttal in 038 provides information on test material and other questions from the original CDFA review.

## GENE MUTATION

### Microbial Systems

\*\*009 019644 (Exhibit 15 of the "Response of the Centre International d' etudes du Lindane to EPA's preliminary Notice of Determination and Position Document 2/3 on Lindane", Volume II) "Bacterial Mutagenicity Tests of Lindane With Mouse Liver Preparations as Metabolizing Systems" (Pharmakologisches Institut Der Universität Mainz, 8/29/80). Lindane (purity unspecified) tested at 0, 15.8, 50, 158, 500, 1580, and 5000 Fg/plate with and without S9 in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 uvrA; duplicate plates; S9 mixes prepared +/- Aroclor induction from NMRI and CF-1 mice; additional assays with TA98 with and without activation and with and without the drug norharman. **NO ADVERSE EFFECT:** no mutagenicity with lindane under any conditions. **ACCEPTABLE.** Schreider 4/29/85, Davis 9/14/88.

014 910384 "Mutagenicity Versus Carcinogenicity of Organochlorine Insecticides (Ames test on Lindane with *S. typhimurium*)" (Public Health Laboratory, Katholieke Universiteit Louvain, 5/4/76) Lindane (purity unspecified) tested at 0, 10, 100 and 1000 Fg/plate with and without NMRI mouse liver S9 in 7 strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538, TA1950 and TA1978); **INSUFFICIENT INFORMATION FOR ASSESSMENT; INCOMPLETE; UNACCEPTABLE** (purity of compound, no statistical analysis, missing individual plate revertant data, some figures unreadable, no quality assurance statement). Schreider 4/24/85.

### Mammalian Systems

\*\*031 034526 "Mammalian Cell (V79) Mutagenicity Test on Lindane (Fetal Hamster Lung Cells)" (Celamerck, 5/28/84) Lindane (purity unspecified) tested at 0.5 to 500 Fg/ml (10 concentrations) with Aroclor 1254 induced male CD-1 mouse S9 and 0.5 to 250 Fg/ml (9 concentrations) without S9 activation in cultured Chinese Hamster lung cells (V79); 2 hours exposure time with S9 and 24 hours without S9; 10<sup>6</sup> cells/replicate; 6 replicates/dose; 8 days expression time; an increase of mutant frequency was observed at 2.4 and 100 Fg/ml in an activated trial but was not confirmed by two subsequent activated trials; NO ADVERSE EFFECT; COMPLETE; ACCEPTABLE. Gee 9/26/85.

EPA one-liner: Negative in V79 cells for mutation to HGPRT deficiency up to levels of toxicity and/or solubility, with or without activation. However, inappropriate S9 microsomes used (from CD-1 mice) instead of sensitive CF-1 mice.

Note: A letter of 7/1/85 from the EPA Product Manager in Volume 031 indicates that the appropriate activation system was from CF-1 mice since that was the strain in which Thorpe & Walker (CDFA Record 059701) demonstrated liver oncogenicity. EPA was convinced that "CD-1" was a typographical error and that CF-1 mice were in fact used. Therefore, the CORE Grade was changed to "Acceptable".

#### CHROMOSOMAL ABERRATIONS

\*\* 133-059 089027 "Mutagenicity Test on Lindane (Technical). In an In Vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells with Multiple Harvests under Conditions of Metabolic Activation." (Murk, H., Hazleton Laboratories America, HLA Study No. 12024-0-437C, 6/5/90). Lindane technical, Lot# DA-433, 99.7% by weight, was tested for induction of chromosomal aberrations with CHO-WBL cells. Concentrations for non-activated cultures were 0 (negative and DMSO), 38.1, 50.8, 76.1, 102 and 152 (toxic) Fg/ml with a 20 hour harvest. For activation with Aroclor 1254-induced male rat liver S9, three harvest times were used -10, 20 and 30 hours following a 2-hour treatment. Concentrations scored were: 10 hours - 25, 49.9, 74.9 and 99.8 Fg/ml; 20 hours - 25.4, 50.8, 76.1, 102 and 152 (toxic) Fg/ml; 30 hours - 99.8 and 150 (toxic) Fg/ml. No induction of chromosomal aberrations was reported. **ACCEPTABLE.** Gee, 12/26/90.

031 034524 "*In vivo* Sister Chromatid Exchange Assay in CF-1 Mouse Bone Marrow Cells with Lindane (oral application)" (Research & Consulting Company, 6/20/84) Lindane (99.8%) tested at 0, 2, 10 and 50 mg/kg in male and 0, 1.6, 8 and 40 mg/kg in female in oleum arachidis by single oral gavage (10 ml/kg) in Charles River CF-1 mice; doses were 1/75, 1/15 and 1/3 of the LD50; 5 mice/sex/group; BUdR tablets implanted 2 hours after dosing; mice sacrificed 24 hours after dosing; 30 metaphases/animal scored; the high dose male SCE frequency was statistically higher than control frequency while the high dose female SCE frequency was statistically lower than control frequency; the averaged frequency was not different from the controls; **NO ADVERSE EFFECT; UNACCEPTABLE-dose** levels not high enough. Originally considered acceptable (Gee 9/25/85); second review considered unacceptable (Gee & Choy 12/10/86); review of C.I.E.L. rebuttal of 9/21/87, still unacceptable (Davis & Gee 9/16/88).

EPA one-liner: Negative in CF-1 males and females for sister-chromatic exchanges at single oral dose up to one third of reported LD50. However, insufficient dosage and sampling sizes employed; no clinical or cytotoxicity at any dose.

Note: A letter of 1/1/85 from the EPA Product Manager in Volume 031 indicates that the CORE Grade has been changed to "Acceptable".

**031 034525** "*In Vivo* Sister Chromatid Exchange Assay in CF-1 Mouse Bone Marrow Cells with Lindane (i.p. injection)." (Research and Consulting Company, 7/17/84). Lindane (99.8%) tested at 0, 1.3, 6.4 and 32.1 mg/kg in oleum arachidis by a single i.p. injection (10 ml/kg) in Charles River CF-1 mice; doses were 1/75, 1/15 and 1/3 of LD50; 5 mice/sex/dose group; BUdR tablets implanted 2 hours after dosing; mice sacrificed 24 hours after dosing; 30 metaphases/animal scored; increases of SCE frequency in female mice at 32.1 mg/kg (1/3 of LD50); **Possible adverse effect, unacceptable** (pending historical control data). Originally considered acceptable (Gee 9/25/85); second review considered unacceptable (Gee & Choy 12/10/86); review of C.I.E.L. rebuttal of 9/21/87, still unacceptable (Davis & Gee 9/16/88). EPA One-Liner: Acceptable, 1/23/89.

#### DNA DAMAGE

\*\* 133-059 089026 "Mutagenicity Test on Lindane (Technical). In the *In Vitro* Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay." (Cifone, M. A., Hazleton Laboratories America, HLA Study No. 12024-0-447, 9/17/90) Lindane technical, Lot# DA-433, 99.7% by weight, was tested with Fischer 344 adult male primary rat hepatocytes. Cells were incubated for 18.8 hours with 0 (DMSO), 0.25, 0.50, 2.5, 5.0, 10 or 15 Fg/ml with 2-AAF as positive control. Triplicate coyer slips for UDS by autoradiography and duplicates for viability by dye exclusion were scored. No evidence of induction of unscheduled DNA synthesis. **Acceptable**. Gee, 12/24/90.

045 067906 "Chemically-Induced Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures: A Comparison With Bacterial Mutagenicity Using 218 Compounds," (Probst, G. S. et al., Lilly Research Laboratories, 8/1/80; Published in *Environmental Mutagenesis* 3:11-32, 1981. Lindane (purity and grade unspecified), was used in an autoradiographic unscheduled DNA synthesis assay with primary hepatocytes (from Fischer 344 rats) at 100 nmoles/ml. Cells were exposed to the compound for 5 hours, and allowed to incubate further for 18-20 hours. Lindane was also used in a modified Ames assay using G46, TA1535, TA100, C3076, TA1537, D3052, TA1538 & TA98, as well as 2 strains of *Escherichia coli* (WP2 & WP2uvrA-) with and without metabolic activation. Four gradient plates provided a 10-fold concentration range/plate (10,000-fold concentration range for the test). **No adverse effect indicated**. Lindane was negative in both the UDS and Ames assay. **Unacceptable** (not a Guideline study, no data presented for Ames assay; only one dose used in the UDS assay; purity and grade of lindane not provided) and **not upgradeable**. Previously reviewed by Davis, 9/11/88. Current reviewer concurs with Davis. M. Silva, 1/12/90.

045 067907 "The Relevance of Covalent Binding to Mouse Liver DNA to the Carcinogenic Action of Hexachlorocyclohexane Isomers" by Sagelsdorff, Lutz, & Schlatter, Institute of Toxicology, ETH & University of Zurich; Publication in *Carcinogenesis* 4:1267-1273, 1983. Hexachlorocyclohexane (HCH) isomers = alpha (98%), gamma (92%), delta (95%) and beta (96%) purity (contamination = epsilon HCH). NMRI, CF1, and B6C3F1 male mice (2 pools/strain/isomer with 2 mice/pool) were dosed by oral gavage with [<sup>3</sup>H]-labeled alpha (6.2-8.5 mg/kg), beta (7.3-7.7 mg/kg), delta (6.8-7.1 mg/kg), or gamma (12-13 mg/kg) HCH. Gamma HCH was also used at 8.7 and 16.7 mg/kg in NMRI, 21 mg/kg in CF1 and 21.6 and 23 in B6C3F1 (2 pools/strain with 2 mice/pool). Animals were terminated after 10 hours and the specific activity of liver DNA was assayed. A time dependent experiment was performed where male NMRI mice (2 pools/time point/isomer with 2 mice/pool) were gavaged with either alpha or gamma [<sup>3</sup>H]-HCH,

then sacrificed after 10 hours or 1, 3, 5 or 10 days. Unscheduled DNA synthesis (UDS) was also examined in NMRI 3.5 hours after injection of  $^{14}\text{C}$ -thymidine and 6.5 hours after gavage of alpha and gamma [ $^3\text{H}$ ]-HCH (2 pools/isomer with 2 mice/pool). Covalent binding to liver DNA was found at equivalent levels for all isomers except beta which had little binding. Gamma HCH binding was equivalent in all three mouse strains. DNA binding of gamma HCH increased over time and UDS was stimulated. No adverse effect. Although covalent binding of lindane to DNA was observed, it was not significant when compared to that of known chemical carcinogens such as aflatoxin. Previously reviewed as **unacceptable** (not a guideline study; review by Davis 9/13/88). The current reviewer agrees. M. Silva, 1/17/90.

045 067908 "Pesticide Induced DNA Damage and Its Repair in Cultured Human Cells" by Ahmed, Hart, & Lewis, Ohio State University (Mutation Research 42:161-174, 1977) Lindane (purity unknown) along with 13 other pesticides was tested for unscheduled DNA synthesis (autoradiography) in SV-40 transformed human fibroblasts (cell line VA-4) at 0 (vehicle = acetone), 1 and 1000 FM (290 Fg/ml), +/- S9 for 8 hours (30 cells scored/coverslip). **No adverse effect indicated.** Lindane showed no increase in UDS. Originally reviewed as a supplemental study (Davis 9/13/88), the current reviewer considers the study **unacceptable and not upgradeable** (not a guideline study; too many deficiencies). M. Silva, 1/17/90.

**NOTE:** The above three studies (045 067906-067908) were considered by EPA to be acceptable studies (1/23/89).

#### NEUROTOXICITY (Not required at this time.)

#### REVIEW ARTICLES AND RELATED SUBMISSIONS

133-066 124419 (Prepared by SRA International, Inc., Washington, D.C.) "Lindane: A review of the scientific literature relative to carcinogenic potential in humans and laboratory animals", 6/17/93. This review discusses available rat and mouse oncogenicity data, considering study outcomes and study limitations including test article identity (such as whether test article was technical lindane vs. other isomers of hexachlorocyclohexane) and appropriateness of dose levels (investigators questioned, for instance, the use of studies which appeared to exceed the MTD). Investigators concluded that the data on lindane indicate a "category E" designation for oncogenic potency (No evidence for carcinogenicity). No new data were presented, hence no worksheet is appropriate. Aldous, 11/24/98.

133-066 124420 Carlton, B. D. and A. M. Blacker "Review of the carcinogenic potential of lindane", June, 1993. These investigators came to the same conclusions as author(s) of the preceding review, namely that lindane should be given a "category E" designation for oncogenicity. No new data were presented, hence no worksheet is appropriate. Aldous, 11/24/98.